CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Imazalil

SB 950-281 Tolerance # 413
Chemical code # 002084

November 21, 1986
Revised 07/28/87, 03/15/88, 12/12/89, 10/02/90, 08/23/91, 11/22/93, 11/10/95

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, possible adverse effect
Teratology, mouse[1]:	No data gap, possible adverse effect
Gene mutation:	No data gap, no adverse effect

Chromosome: No data gap, no adverse effect

DNA damage: No data gap, no adverse effect

Neurotoxicity: Not required at this time

----------Toxicology one-liners are attached.

** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T951110
Revised by Gee, 10/02/90; M. Silva, 8/23/91, 11/22/93, 11/10/95

Rectified with the DPR Library printout through document 413-092, Record #’s 139659 and 900000+.

1 - There is also an unacceptable rabbit teratology study on file which did not show adverse effects.

NOTE: These pages contain summaries only. Each individual worksheet may contain additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

Note: Studies were conducted with imazalil base (R23979), sulphate (R27180) or nitrate (R18531). No studies apparent for acetate (R47657). Information from DPR document No.'s 413-009, 413-032 and 413-031 (especially record # 024343) were useful. From the chemistry and metabolism submitted, the properties of the sulfate are quite similar to the free base in terms of solubility. According to record 024343, when the imidazole ring is protonated, the solubility in water is increased. The counter ion (nitrate, sulfate, acetate) is stated to play no role in equilibrium or in absorption of the base. In addition, the acute oral toxicity of the free base and the salts are approximately the same. Although the registered product is imazalil itself, it would seem acceptable to use one of the other forms as long as the study specifically addressed the doses actually used in terms of the free base. Gee, 11/8/89.

CHRONIC TOXICITY, RAT

The collective data from all the studies (chronic and oncogenicity: 010/982436, 010/982435, 011/982442, 011/982444, 058/074001, 039/047213 and 041/047215) have been reviewed. It is evident that the liver is the primary target organ at doses of $\geq$ 400 ppm. Although none of the studies are complete and acceptable in and of themselves, the collective data are sufficient to fill the chronic rat data gap. (M. Silva, 8/23/91)

010, 050 982435, 051388 "Oral Toxicity Study in Wistar Rats" (Janssen, no. 480, 8/30/75) Imazalil, R 23979, no purity stated, batch no. A 18/1; mixed as a 50% powder in feed and fed at 0, 5, 20 or 80 mg active compound per 100 gm food, in diets prepared weekly; 10/sex/group for 2 years, with additional 10/sex/group also sacrificed at 6 and at 12 months; Wistar rats;

No adverse effect; NOEL possibly 20 mg/100 gm food (approximately 3 to 4 mg/kg b. wt. in males and 3.8 in females) based on slight increase in liver and kidney weights and slight liver changes. Unacceptable (inadequate number of animals at risk, marginal toxicity, no purity for test material, no individual histopathology data.) A 4-week study (#982424 in volume 010) showed no significant effects on mortality or body weight at 200 mg/kg given by gavage on 6 days/week. 982435 is the full report-reviewed by Apostolou 7/22/85; 051388 is a summary of rat feeding studies-reviewed by Davis 7/17/87. Supplemental review by Gee, 11/8/89. Not upgradeable.
EPA 1-liner: Supplementary. Systemic NOEL = 3 mg/kg (male); Systemic NOEL = 3.8 mg/kg (female) (liver weight increase).

039, 040 047213, 047214 "Eighteen-month Oral Toxicity Study with Imazalil Base-R 23979 in Rats". (CIVO institutes TNO, The Netherlands, Report No. V84.220555, 5/84) Imazalil technical, batch D 41/03, 98.1%, fed in the diet for 18 months to Cpb:WU rats, 20/sex/group at 0, 25, 100 or 400 ppm; No adverse effect; sys NOEL = 100 ppm (decreased body weight gain in females, liver changes in males), Unacceptable (length of study, dose selection for a chronic study - should show distinct signs of toxicity. Oncogenicity study at these dose levels was considered acceptable.) (Gee 11/5/86)

057 073999 Rebuttal for 047213 discussing the length of the study in terms of OECD guidelines. See DPR response of 12/12/89. (Gee, 12/89)

066 096404 Rebuttal for 047214-214, includes abnormal findings observed in toxicity studies in rodents (subchronic, chronic, carcinogenicity and segment I, II and III reproduction studies). No worksheet. M. Silva, 8/15/91.

069 098200 "The Biological and Toxicological Properties of Imazalil," Thienpont, D., Van Cutsem, J., Van Cauteren, H. and Marsboom, R. (1981), Janssen Pharmaceutica, Research Laboratories, Beerse (Belgium). Published in: Arzneim-Forsch./Drug Research, 31(1)2, 309-315. This article was a compilation of studies (acute and chronic) performed by Janssen Pharmaceutica, using imazalil (all are on file at CDPR). No worksheet. M. Silva, 8/21/91.

See also supplemental studies at the end of this Summary.

CHRONIC TOXICITY, DOG

** 027, 066 090214, 096400 "Chronic Toxicity Study in Beagle Dogs, Repeated Dosage for 12 Months". (Dept. of Toxicology, Janssen Research Foundation, Experiment No. 1899, 11/6/89) Imazalil base, R23979, 97.2%, was given to beagle dogs by gelatin capsule on a daily basis for 1 year, at 0, 1.25, 2.5 or 20 mg/kg/day, 4/sex/group. NOEL = 2.5 mg/kg/day (slightly softened feces, salivation, vomiting, decreased appetite, body weight gain reduced, increase in alkaline phosphatase, increased liver weight with no histological changes.) **No adverse effects.** Previously reviewed as unacceptable **(**D. Shimer and Gee, 10/1/90), upon receipt and review of the stability data (096400), the study has been upgraded to acceptable. M. Silva, 8/15/91.

010 982434 "Oral Toxicity Study in Beagle Dogs". (Janssen, no. 370, 4/12/77). Imazalil, R 23979, > 95% purity; given by oral capsule to 3/sex/group for two years at 0, 1.25, 5 or 20 mg/kg; no chronic effects reported; nominal systemic NOEL = 1.25 (decreased body weight gain at 5 and 20 mg/kg); Unacceptable (3 of 4 recommended number of animals, no individual histopathology results - only a check list of tissues, no individual clinical observations, no food consumption although report states decreased food intake at high dose.) No consistent adverse effect. (Apostolou 7/22/85)
EPA 1-liner: Minimum. NOEL = 1.25 mg/kg (decreased body weight.)

031 024995. Addendum to 010 982434. Statistical analysis of weight gain at the end of study.

ONCOGENICITY, RAT

011 982444 "Oral carcinogenicity Study in Wistar Rats" (Janssen Pharmaceutical, No. 667, 4/20/79) Imazalil, R23979, wettable powder of 25% aerosil, 25% starch and 50% a.i.; fed to Wistar rats, 50/sex/group, at 0, 2.5, 10 or 40 mg/kg/day, 2 years; oncogenicity NOEL $\geq$ 40 mg/kg/day; Unacceptable (dose selection not justified, stability not included, animals were 3 months at initiation of study, missing individual data on body weights, food intake, inadequate tissues for histopathology and no organ weights, inadequate number of survivors at term.) Not upgradeable. (Apostolou 7/17/85)

EPA 1-liner: Minimum. Oncogenic NOEL > 40 mg/kg (HDT).

** 041 to 046 047212, 047215-047219 "Lifespan Oral Carcinogenicity Study with Imazalil Base-R 23979 in Rats (Final Report)". (CIVO institutes TNO, The Netherlands, 11/85) Imazalil technical, 98.1%, fed in the diet at 0, 25, 100 or 400 ppm to 50 Cpb:WU rats/sex/group for 30 months; eye exams at weeks 52 and 104; nominal sys NOEL = 100 ppm (slight body weight effect), no hematology or urinalysis, clinical chemistry of albumin only so cannot be considered for a chronic study; nominal oncogenicity NOEL ≥ 400 ppm - no evidence. Acceptable. (Gee 11/5/86.)

057 074000 Summary table and individual histopathology for non-tumorous changes in the 30-month rat study above. No worksheet. (Gee, 12/12/89)

ONCOGENICITY, MOUSE

Subchronic Study:

080, 082 129859, 132556 "Three-Month Oral Mechanistic Toxicity Study With One Month Interim Sacrifice in SPF Albino Swiss Mice," (Van Deun, K., Experiment #: 3140, Janssen Pharmaceutica, N.V., 2/18/94). Imazalil technical (purity = 98%) was fed in diet to SPF Albino Swiss mice at 0, 50, 200 and 600 (40/sex/dose) for 3 months with a 1 month interim sacrifice. NOAEL = 50 ppm A NOEL was not achieved in this study. (Food wastage and food consumption was increased in females at all doses (both interim and terminal sacrifice) although compound intake was decreased but was considered in the report to be due to food palatability. Interim kill showed increased ALP and ALT were increased in both sexes at 600 ppm. At study termination males had increased ALT at 600 ppm. Males at ≥ 200 ppm and females at 600 ppm showed increased absolute and relative liver weights (1 & 3 months). A significant increase in dark livers occurred in both sexes at 600 ppm (1 & 3 months). Both sexes at ≥ 200 ppm showed increased centrilobular clearer aspect and increased small and large vacuoles in liver (1 & 3 months). Males at 600 ppm had increased Kupffer cell prominence (3 months) and hepatocytic necrosis (1 & 3 months).) Possible adverse effect indicated. These data are supplemental. M. Silva, 10/24/95.

Oncogenicity Study:

**** 078, 079, 081, 082 & 092 126581, 129859, 129860, 132556, 139650, 139659** "Carcinogenicity Study in Swiss Mice," (Verstraeten, A., Janssen Research Foundation, Beerse, Belgium; Experiment #: 2194, 10/13/93). Imazalil technical (R 23979, batch #'s: 73001, 75801 & 92001, purity = 93%) was administered in diet to SPF Swiss mice (50/sex/group) at 0 (vehicle = 50:50 Aerosil and corn starch), 50, 200 and 600 ppm for 2 years. **Possible adverse effect.** ONCOGENICITY NOEL = 50 ppm (An increase in hepatocytic neoplasms and hepatic nodules in males at $\geq$ 200 ppm and in females at 600 ppm was observed. Males showed gross liver pathology at 200 ppm.) CHRONIC NOEL = 200 ppm (A significantly decreased body weight and body weight gain was observed in males at 600 ppm. Effects of hematology and serum chemistry parameters was observed in both sexes primarily at 600 ppm. Absolute and relative liver weights were increased in both sexes (primarily males) at 600 ppm. Females also showed decreased weight in lung, kidney & spleen at 600 ppm.) Previously reviewed as unacceptable (Silva, 11/8/93), upon submission and review of the requested information (analysis & purity and homogeneity data for imazalil in diet) the study has been upgraded to acceptable. M. Silva, 10/25/95.

NOTE: Document 078/126581 (reviewed above) contained an adverse effects disclosure for mouse oncogenicity (hepatic neoplasms in males at ≥ 200 ppm & females at 600 ppm). These conclusions are in agreement with the DPR review. M. Silva, 11/17/93.

011 982442 "Oral Carcinogenicity Study in Albino Swiss Mice" (Janssen Pharmaceutical, No. 666, 4/4/79). Imazalil sulphate, R27180, lot A 11/1; > 95%, given orally in the drinking water to 50/sex/group of Albino Swiss mice at 0, 6.25, 25 or 100 ppm equivalent to 2.5, 10 or 40 mg/kg/day based on each mouse drinking about 100 ml/week; 18-month study; nominal oncogenicity NOEL ≥ 100 ppm; Unacceptable (no justification of dose selection, no stability of test article, no organ weights, no body weights, no water consumption, histopathology on only 12 tissues, high mortality, husbandry (25 animals per cage for first 8 months), no analysis of drinking water, unreadable tables of individual findings.) (Apostolou 7/18/85) EPA 1-liner: Minimum. Oncogenic NOEL > 40 mg/kg (HDT).

REPRODUCTION, RAT

**** 074, 083 118468, 132558** "2-Generation Reproduction Study with 1 Litter Per Generation in Wistar Rats," (Van Cauteren, H., Janssen Research Foundation, Beerse, Belgium, 10/26/92, Experiment #: 2337). Imazalil technical (R 23979, 98% pure, batch #: ZR023979 G3A231) was administered in diet to Wistar rats (24/sex/group) at 0, 5, 20 and 80 mg/kg for two generations until weaning of the second generation. Parental Chronic NOEL = 5 mg/kg (Females (& males in P0 only) of both generations showed clinical signs at 80 mg/kg. There was a significant decrease in mean body weights and body weight gain for P0 males during precohabitation & P0 females during pregnancy, birth & lactation at 80 mg/kg. F1 body weights during pregnancy were significantly decreased at ≥ 20 mg/kg body weight & birth & lactation at 80 mg/kg. P0 food consumption was significantly decreased in females at 80 mg/kg & in F1 from day 4-13 of lactation at 80 mg/kg. P0 males showed hepatocytic vacuolization & females showed decreased corpora lutea by histopathology at 80 mg/kg. F1 females showed increased proestrus & metestrus stages, decreased number of corpora lutea generations & clear aspect of interstitial tissues by histopathology at 80 mg/kg.) Reproductive NOEL = 5 mg/kg (P0 & F1

showed an increase in duration of gestation at 80 mg/kg. There was a decrease in litter size (P0 = 80 & F1 ≥ 20 mg/kg) and # litters (P0), # live pups/female & survival rate (day 4-21) & an increase in # dead pups/female at 80 mg/kg.) Pup NOEL = 20 mg/kg (P0 pups (F1 offspring) showed a decreased incidence of pups showing a ringtail at ≥ 20 mg/kg. There was also an increased incidence of pups with bad condition and hypothermia at 80 mg/kg (associated with decreased survival)). **Possible adverse effect** (Increased pup/litter mortality, decreased number of litters and mean litter size). Previously reviewed as unacceptable (Silva, 11/9/93) upon submission of the requested data, the study has been upgraded to acceptable. Silva, 10/26/95.

011 982455 "Oral Three-generation Study in Wistar Rats" (Janssen Pharmaceutica, No. 736, 3/15/78) Imazalil, R23979, Batch no. C 03/1, R. E. 11.108, with approximately 99% purity; fed to Wistar rats, 10 males and 20 females per group in the F0 groups and varying numbers in subsequent generations, at 0, 50, 200 or 800 ppm for 3 generations, 2 litters; dosing schedule of F0 parents began on day 0 of mating rather than 10 weeks prior to mating; subsequent parental animals were fed from weaning onward; on 22nd day of gestation for the F3b litters, the dams were killed and the fetuses examined for developmental affects; parental and reproduction nominal NOEL $\geq$ 800 ppm; unacceptable (dosing schedule, no justification of dose levels [see 982447 where five of 20 died at this high dose level], no analysis of diet, no histopathology of any animals). The initial review noted a decrease in parental pregnancies. This occurred in one mating interval for the F1a litters at the high dose and is not considered toxicologically significant; the decrease in litter size occurred in the F1a only at the high dose and was not reproduced in subsequent litters so is not considered significant; the test material characterization was included; the number of stillborn fetuses/no. of all fetuses was slightly higher in all treated groups compared with controls but no dose relationship was evident, Presentation of the laboratory's historical control data would help in evaluating the significance of this effect. Not upgradeable. (Apostolou 7/19/85, Gee 11/86.)
EPA 1-liner: Minimum. Reproductive NOEL = 40 mg/kg/day (HDT) (80 mg/100 gm food). (2.5, 10 and 40 mg/kg)

TERATOLOGY, RAT

011 982446 "Potential of R 18531 for Embryotoxicity and Teratogenic Effects in Rats Receiving R 18531 Orally" (Report no. 356; Janssen Pharmaceutica; 04/10/70). Imazalil nitrate, batch AO 301, purity not given; 0, 50, 200, or 800 ppm in the diets of 18 or 19 pregnant females/dose on gestation days 6-15 and sacrificed on day 22; no signs of maternal toxicity; no signs of fetal toxicity or developmental abnormalities; no adverse effect indicated; study unacceptable (no analysis of test material, no analysis of dose in dietary mixture, no maternal toxicity, no uterine weights, no rationale for using nitrate salt of

active ingredient, incomplete individual maternal and fetal data); study not upgradeable (dose range too low to produce maternal toxicity); Apostolou, 07/18/85; one-liner updated, no new worksheet. (Morris 01/06/88)
EPA one-liner: Minimum. Terata NOEL $\geq$ 40 mg/kg (HDT) (80 mg/100 gm food), maternal NOEL $\geq$ 40 mg/kg.

**** 059, 063, 066 074002, 088645, 096401-3**, "Embryotoxicity and Teratogenicity Study in Sprague-Dawley Rats", (Laboratoires Janssen, Research Department, France, Experiment No. 2003/88-05, 7/5/88). R 27180 technical grade (imazalil sulfate), purity 99.9%, lot ZR027180G1A631, administered by gavage at concentrations of 0 (water), 40, 80 or 120 mg/kg to 24 mated female Sprague-Dawley rats/group on days 6 through 16 of gestation (day sperm positive = day 1). Nominal maternal NOEL = 40 mg/kg/day (reduced maternal total body weight, decreased body weight change and food consumption at 80 and 120 mg/kg/day). **Possible adverse effect indicated:** Increased mean resorptions per litter and reduced mean fetal weights in the absence of significant maternal effects. Nominal developmental NOEL = 40 mg/kg. Initially reviewed as unacceptable but upgradeable (analyses of dosing solutions; method and frequency of dosing solution preparation; historical control data for resorptions). (Kishiyama, 9/27/89 and Gee, 11/1/89) Record 063 088645 contains the historical control data, satisfying that deficiency. (Gee, 10/2/90). Upon submission of analysis of dosing solutions (066 096401-3), the study has been upgraded to acceptable. Note: a pilot study was submitted (066 096403--separate worksheet). M. Silva, 8/21/91.

063 088645 "Supplemental Data Submission to Toxicology Worksheet (W074002.833)" (Laboratories Janssen, 7/90) The submission consists of historical control data for years 87 - 88 for resorptions and pup weights. In addition, analytical information on the lot of imazalil sulfate used in the rat teratology study is included. No information on analyses of the dosing solutions used to treat the dams has been submitted. The study is still upgradeable with submission of these analyses. No worksheet. (Gee, 10/2/90)

066 096401 Certificate of analysis for imazalil sulphate (batch XZR027180G1A631), mother solution (Agroform 047701), before the start of the study (2/12/88) and certificate of analysis (6/27/90) documenting the storage stability of the mother solution (batch 047701). Dosing sheet of experiment 88-05 was included. No worksheet. M. Silva, 8/16/91.

066 096402 This report contains a storage stability study of a 7.5% dilution (Report #: P013, Rev. 3) demonstrating the stability of imazalil in solution at ambient temperature for 24 months. The highest concentration given in the rat teratology study (exp. 2003, also reported

as 88-05) is 24 ml/L, corresponding to 1.2% w/v of imazalil base. No worksheet. M. Silva, 8/15/91.

073 113031 This volume contains historical control data for rat teratology studies pertaining to mean litter size, mean # of resorptions & mean body weights of live pups at different dose levels in Experiment #: 2003. Control data from this experiment were included in addition to historical control data of other previously conducted studies. These data are supplemental (no worksheet). M. Silva, 11/17/93.

TERATOLOGY, RABBIT

038 047211 "Oral Embryotoxicity and Teratogenicity Study in New Zealand White Rabbits (Segment II)" (Janssen, 4/25/85, 1482) Imazalil, R 18531 (nitrate), 99%, given orally to 15 rabbits per group at 0, 1.25, 2.5 or 5.0 mg/kg, days 6 - 18; developmental NOEL $\geq$ 5 mg/kg, maternal NOEL $\geq$ 5 mg/kg. No developmental toxicity effect reported. Unacceptable (dose selection, only 1/3 of fetuses for visceral examination). Not upgradeable. (Gee 11/5/86)

TERATOLOGY, MOUSE

Note: EPA 1-liner list contains reference to another teratogenicity study in mice dated 11/28/83 by Janssen, No 657, which is not on file at DPR. Core grade: Supplementary. (Remsen (Gee) 3/6/86.).

**** 075, 084 119314, 132539** "Embryotoxicity and Teratogenicity Study in Albino Mice," (Dirkx, P., Janssen Research Foundation, Beerse, Belgium, 12/1/92, Experiment #: 2712). Imazalil sulphate (R27180, Batch #: ZR027180 PUA 631, purity = 98.2%) was administered by gavage to mated Cobs CD1 mice (30/dose) at 0 (vehicle = deionized water), 10, 40, 80 and 120 mg/kg during days 6-16 of pregnancy. **Maternal NOEL =** 40 mg/kg (There was an increase in mortality and clinical signs at ≥ 80 mg/kg. Body weight and body weight gain were significantly decreased at ≥ 80 mg/kg.) **Developmental NOEL =** 10 mg/kg (There was an increase in resorbed fetuses and resorptions/litter at ≥ 40 mg/kg. The ratio of live fetuses/implantations & litter size/implantations was significantly reduced at 80 mg/kg, but began to show a trend at 40 mg/kg. There was an increase in extra 14th pair of ribs at ≥ 80 mg/kg and an increase in rudimentary sternum bones, sternum bones cleaved and incomplete ossification of sternum bones at 120 mg/kg.) Previously reviewed as unacceptable (Silva, 11/2/93) upon submission and review of requested information (analysis of imazalil technical & dosing material, description of temperature and humidity conditions, description of randomization process for placing animals in the dosage groups & GLP statement), the study has been upgraded to acceptable. **Possible adverse effect indicated** (increased fetal effects and embryotoxicity in the absence of equally severe maternal toxicity at ≥ 40 mg/kg). Silva, 10/26/95.

**** 068 089555** "Embryotoxicity and Teratogenicity Study in Cobs CD1 Mice," (Levron, J.C., Van Cauteren, H., Sanz, G., and Marsboom, R., Research Department, Laboratoires Janssen, Aubervilliers, France, 1991). Imazalil Sulfate technical (R 27180; batch #: PUA 521; 99% pure) was administered by gavage at 0 (water), 10, 40, 80 or 120 mg/kg/day to mated female Cobs CD1 mice (30/group) during days 6-16 of pregnancy (# days after copulation). **Maternal NOEL =** 10 mg/kg/day (Increased mortality was reported at 120 mg/kg. Decreased body weight, body weight gain and food consumption was reported at ≥ 40 mg/kg/day.) **Developmental NOEL** <

10 mg/kg/day (Decreased number of live fetuses, increased resorptions and decreased weights of fetuses was observed. An increase in developmental effects in the 14th rib was also observed.) **Possible adverse effect** (Increased resorptions, decreased live fetuses and fetal body weights, as well as increased developmental effects in 14th rib were reported.) Acceptable, M. Silva, 8/5/91.

037 036068 "Embryotoxicity and Teratogenicity Study in COBS Mice (Segment II)" (Lab. Janssen, no. 85-02, 3/25/85). Imazalil (lot C 3001 of R 27180 sulfate), 101.0% purity) given orally to 24 COBS mice per group, days 6 to 16 of gestation, at 0, 2.5, 10 or 40 mg/kg b. wt.; NOEL $\geq$ 40 mg/kg/day; unacceptable (missing analytical certificate, no justification of dose selection, no signatures, no evidence approached MTD,) possibly upgradeable. (Gee, 3/6/86)

053 057757 "Embryotoxicity And Teratogenicity Study In Cobs CD1 Mice (Segment II)", (R 27180, Experiment No. 86-07; Laboratoires Janssen, Aubervillers, France; 09/18/86). Imazalil sulfate, 98.9 - 99.7%; 0, 40, 80, or 120 mg/kg/day by oral gavage (1% Tween 80 as vehicle) on days 6 - 16 to 30 females/dose and sacrificed on day 19; nominal maternal NOEL = 40 mg/kg (decreased maternal weight gain and food consumption at 80 and 120 mg/kg), nominal developmental NOEL < 40 mg/kg (dose-dependent decrease in mean live litter size and increased resorptions at 40, 80, 120 mg/kg); **possible adverse effect - developmental NOEL < maternal NOEL**; study unacceptable (no rationale for vehicle, dosing formulation not analyzed, < 20 litters at 80 and 100 mg/kg, fetuses not cleared and stained, fetal NOEL not established); study not upgradeable. (Morris/Gee, 12/14/87 and Gee, 10/2/90)

056 070269 Rebuttal by the registrant to the finding of a possible adverse effect in the above study. See response of DPR dated December 12, 1989, for comments. No change in status at this time. (Gee,12/12/89)

072 112984 This volume contains a protocol for "Segment II: Oral embryotoxicity and teratogenicity study in COBS CD1 mice." No data are included and this information is supplemental (no worksheet). M. Silva, 11/22/93.

Comment: No adverse effects were observed in the study with the high dose of 40 mg/kg/day, however an MTD was not reached. In the studies performed at higher doses (including an acceptable study), adverse effects were reported. The same, or similar effects were also reported in rat teratology study: 059 074002 and therefore imazalil will be considered to have adverse treatment-related effects for mouse and rat fetuses. M. Silva, 11/10/95.

GENE MUTATION

** 037 036069 "In vitro Mutagenicity Screening of Imazalil, McN-JR-23979, by Microsomal Activation Bacterial Assays" (McNeil Laboratories, No. 515 (770914), 9/15/77) Imazalil, no purity stated; Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100; tested at 0, 0.5, 5,

10, 20 or 30 ug/plate; duplicate or triplicates, 2 trials, with and without mouse liver activation; no increase in reversion rate, 50 ug/plate stated to be bacteriostatic. Acceptable with minor variations (no positive controls for -S9). (Remsen (Gee) 3/6/86.)

037, 050 036070, 051390 "Sex-Linked Recessive Lethal Test in Drosophila melanogaster". (Janssen Pharmaceutica, Exp. No. 1146, 9/7/82) Imazalil (99.0% purity, batch D 5701) fed in 5% saccharose solution for 3 days to Berlin K males at 0, 250, or 1000 ppm; 100 males/group mated 1:3 to Muller 5 females in three broods (3-2-2 days); two tests run with no evidence for mutagenicity; Complete, unacceptable - too few chromosomes tested. (Gee, 3/6/87, Green and Davis 7/28/87)

CHROMOSOME

** 060, 063 074003, 088646 "Report on the In Vitro Chromosome Aberration Assay on Human Lymphocytes", (S.C.K./C.E.N., Belgium, Exp. No. - 833, 10/20/86, revised 6/27/90). R 23979, purity 98.3%, at concentrations of 0 (DMSO), 50, 200, 400 or 800 µg/culture of 5.5 ml total volume (equivalent to 9, 36, 73 and 145 µg/ml) was tested with human peripheral blood lymphocytes (as whole blood) from two "healthy male donors" in the presence of rat liver (S9) activation (three pooled livers from phenobarbitone/Aroclor-induced male Wistar rats) for 2 hours and without S9 activation for 24 hours. No adverse effect indicated - no dose related increase in chromosomal aberrations. Initially reviewed as Unacceptable, possibly Upgradeable (report lacked data on cell survival, gaps were included in incidence tables, data in summary tables versus raw data did not correlate; supporting evidence for a single harvest time). Only 25 scored per sample for a total of 50 per concentration. (Kishiyama, 9/21/89 and Gee, 10/30/89) The revised report addressed the major concerns and the study is upgraded to acceptable status. (Gee, 10/2/90)

011 982464 "Micronucleus Test in Rats" (Janssen Pharmaceutical, No. 916, 12/18/79) Imazalil, R23979, no purity stated in report but response (47208, volume 413-038) states 100%; rat micronucleus test with exposure of 6 males per group to 0, 10, 40 or 160 mg/kg by i.p. injection given twice at 24 - hour intervals and sacrifice 6 hours after second dose; scored 2500 erythrocytes per slide and per animal; unacceptable (single sampling time, use of only males without justification). Response to review contains purity of test article, indication that with 2/6 deaths in high dose group, an adequate level was used and is acknowledged as such. Response includes a statement that a quantitative difference may exist between sexes but a qualitative one is not likely. Neither the report nor the response provide the comparative acute LD50 values between sexes. Data from another document submitted earlier by the registrant indicates the oral LD50 for female rats is 227 mg/kg and for males, is 343 for the base form with the LD50’s for the other forms (nitrate, sulphate and acetate) being approximately 85% that of males. The objection of a single sampling time prevents the study from being upgradeable to acceptable. (Apostolou 7/22/85, Gee 11/5/86.)
EPA 1-liner: Minimum. No structural chromosome mutations induced at 160 mg/kg (HLT).

038 047208 (Janssen, 6/6/86) Response to review of 982464. Use of males only is discussed in terms that it is unlikely a qualitative difference would exist between sexes. The single sampling time remains a serious objection. Study remains unacceptable.

011 982465 "Dominant Lethal Test In Male Mice (Single Oral Dose)", (R 23979, Exp. No. 649 ; Janssen Pharmaceutica N.V., Belgium; 04/06/76). Imazalil, 98.8 - 101.2%; 6 males/dose given 0, 10, 40, or 160 mg/kg by oral gavage and housed with 4 undosed females/male for 1 week for 8 successive weeks; females necropsied 15 days after start of mating period; cyclophosphamide (210 mg/kg) as positive control; no dose-related toxicity in males; large variance in fertility attributed to pseudopregnancies in females not dose-related; no dose-related changes in pre- or post-implantation effects; no adverse effect indicated; study unacceptable (no dose-related male toxicity, inadequate explanation of variance in fertility, inadequate number of pregnant females, no distinction between early and late implantation loss); study not upgradeable; initially reviewed as having a possible adverse effect but rereview determined that it was not a dose-related effect. (Apostolou, 7/22/85, Gee, 11/5/86, Morris/Gee, 12/14/87)
EPA 1 liner: Minimum. Not a mutagen at 160 mg/kg (HDT).

038 047209 (Janssen, 6/6/86) Response to review of 982465. Contains purity of imazalil as 98-101%. The document contains an argument that the reduced pregnancy rate, especially in the first week, "gives evidence for our supposition of induced toxicity" and is used as an argument for dose selection. Because there is no increase in preimplantation loss, this argues against post-meiotic effects.

053 057755. Revised report of 413-011; 982465. Adverse effect status changed from "possible adverse effect indicated" to "no adverse effect indicated." (Morris/Gee, 12/14/87)

011 982467 "Dominant Lethal Test In Female Mice (Single Oral Dose)", (R 23979, Exp. No. 650; Janssen Pharmaceutical N.V., Belgium; 04/08/76). Imazalil, 98.8 - 101.2%; 50 females / dose given 0, 10, 40, or 160 mg/kg by oral gavage and housed with 1 undosed male / female for 5 days; females necropsied on pregnancy days 11 - 17; cyclophosphamide (210 mg/kg) as positive control; decreased fertility at 160 mg/kg; no dose-related changes in pre- or post-implantation effects; no adverse effect indicated; study unacceptable (inadequate response with positive control, inadequate explanation of low fertility, incorrect distinction between early and late implantation loss); initially reviewed as having a possible adverse effect but

rereview determined that it was not a genotoxic effect. (Apostolou, 7/22/85, Gee, 11/5/86, Morris/Gee, 12/14/87)
EPA 1 liner: Minimum. Not a mutagen at 160 mg/kg (HDT) (female).

038 047210 (Janssen, 6/6/86) Response to review of 982567, 7/22/85. Purity of test article used in 9825657 stated to be 99 - 101 %. Reference for test procedure is given and dose selection is defended based on decreased mating as a sign of toxicity. The document indicates a newly hired technician made incorrect distinctions between early and late deaths in the study and only total fetal death is meaningful. (Gee 11/5/86)

053 057756. Revised report of 413-011; 982467. Adverse effect status changed from "possible adverse effect indicated" to "no adverse effect indicated."

DNA DAMAGE

037, 050 036071, 051389 "SOS Chromotest in Escherichia coli", (Janssen, Experiment No. 1395, 5/6/84) Imazalil = R 23979 (99.6% purity, lot D3801) at 0, 6.25, 12.50, 25, 50, 100, 200, or 400 ng/well for 3 hours with activation and 2 hours without activation using E. coli in which the gene for beta-galactosidase is linked to the SOS operator gene; no adverse effect or cytotoxicity is reported; Unacceptable-dose levels may not have been high enough. Remsen 3/6/86; supplementary material in 051389 reviewed by Green and Davis 7/28/87.

** 061 074004 "Micronucleus Test in Mice", (Janssen Pharmaceutica N.V., Belgium, Exp. No. 1911, 2/29/88). R 23979, purity 98.5%, administered orally in a single dose at concentrations of 0 (propylene glycol), 0 (cyclophosphamide), 20, 80 or 320 mg/kg body weight to 5 Albino Swiss mice/sex/group. Sacrifice times at 24, 48 and 72 hours post-treatment for all groups except the positive (cyclophosphamide) control with sacrifice at 48 hours. Scored a total of 1000 polychromatic and normochromatic erythrocytes per animal. There were 2, 4 and 4 deaths in the high dose groups with sacrifice time at 24, 48 and 72 hours - both sexes. The % polychromatic erythrocytes decreased significantly in both sexes at 320 mg/kg body weight, indicating cytotoxicity to the bone marrow and adequate dosing. In addition, there was greater weight loss in the high dose group compared with solvent controls. Negative for an adverse effect: The number of micronucleated polychromatic erythrocytes did not increase with imazalil treatment. Acceptable. (Kishiyama, 9/25/89 and Gee, 10/31/89.)

NEUROTOXICITY

Not required at this time.

SUPPLEMENTAL

Comment: The following are studies with possible relevance to hazard assessment, however the study designs do not conform to FIFRA guidelines. (Morris/Aldous, 3/88)

066 096403 "Dose-Range Finding: Segment II Pilot Study in Non-Pregnant Sprague Dawley Rats," (Gillardin, J.M., Van Cauteren, H., Sanz, G., and Marsboom, R., Research Department, Laboratoires Janssen, France, 6/22/88). Imazalil sulfate (Batch #: Agroform 047701, 99.7-100.6% pure) was administered to virgin Sprague-Dawley rats by gavage at 0 (water), 80, 120 and 160 mg/kg for 8 days (10/group). NOEL = 120 mg/kg (Mortality increased, food consumption decreased, and water consumption increased significantly at the high dose. Females showed hypothermia, ptosis and piloerection at 160 mg/kg.) The MTD was exceeded at the high dose, as indicated by mortality. **Supplemental. M. Silva, 8/16/91.**

011 982447 "Oral Embryotoxicity and Teratogenicity Study in Wistar Rats During the Peri- and Postnatal Study (Segment III)" (Experiment No. 597; Janssen Pharmaceutica Research Laboratories, Beerse, Belgium; 08/28/75). Imazalil nitrate (R 18531), batch A 10/2, purity not given; 0, 50, 200, or 800 ppm in the diets of 20 pregnant females/dose on pregnancy day 16 through lactation week 3; nominal maternal NOEL = 200 ppm (5/20 dams died at 800 ppm), nominal fetal NOEL = 200 ppm ([live births]/[total births] = 0.72 at 800 ppm vs 0.96 to 0.99 for other groups); **possible adverse effect indicated, reproductive toxicity, neonatal NOEL = 50 ppm (% pup survival at 4 days postpartum is 55% at 800 ppm, 86% at 200 ppm, 95% at 50 and 0 ppm);** study not evaluated for acceptability because it does not conform to any SB-950 test type (exposure not done during organogenesis, no prepartal necropsies of dams and fetuses), study deficient in general areas (no analysis of test material, no analysis of dose in dietary mixture, no rationale for using nitrate salt of active ingredient, incomplete individual maternal and pup data); originally evaluated as a teratogenicity study by Apostolou, 07/18/85 and Gee, 11/86 changed to not evaluated, special toxicological testing by Morris/Parker, 01/14/88.
EPA one-liner: Minimum. Terata NOEL = 40 mg/kg/day (HDT), maternal toxic NOEL = 10 mg/kg/day (decreased food consumption, increased mortality). Fetotoxic NOEL $\geq$ 40 mg/kg/day. Dosage levels = 5, 20 and 80 mg/100 gm food or 2.5, 10 and 40 mg/kg/day.

Comment: The rat teratology study (DPR doc. # 413-011, rec. # 982446) did not indicate an adverse effect while the supplemental study (DPR doc. #413-011, rec. #982447) did indicate a possible adverse effect. These findings are not in conflict because the exposure protocol in the first study was designed to detect developmental effects occurring during organogenesis while the exposure protocol in the second study was designed to detect post-organogenic developmental and postnatal effects. In the second study, a developmental adverse effect was not indicated because the maternal NOEL (200 ppm based on lethality at 800 ppm) equalled the fetal NOEL (200 ppm based on increased stillbirths at 800 ppm). However the second study did indicate a **possible reproductive adverse effect with a NOEL of 50 ppm** based on decreased pup four-day-survival at 200 and 800 ppm. (Morris/Aldous, 1988)

010 982424 "Cumulative Oral Toxicity in Wistar Rats (repeated dosage for 4 weeks)," (Experiment No. 644; Janssen Pharmaceutica Research Laboratories, Belgium; 03/18/76) Imazalil, R23979, batch #B 0302; 0 (vehicle not stated), 100, or 200 mg/kg by oral gavage, 6 days/week for 4 weeks, to 10 Wistar rats/sex/dose; animals observed for 2 weeks past dosing period; prior to dosing, eyes were examined and again at termination; no dose-related clinical signs or mortality; no pathology or histology data included in the report; no adverse effect indicated; study not evaluated for acceptability because it does not conform to any SB-950 test type; study deficient in general areas (no analysis of test material, no pathology data); no worksheet. (Morris/Davis, 1/15/88). Supplemental review by Gee, 11/8/89.

010 982436 Untitled, Report No. 342; Janssen Pharmaceutica Research Laboratories, Belgium; 07/20/72. Imazalil nitrate, R18531, batch # A 03/1, 99 - 100%; 0, 50, 200, or 800 ppm in the diets of 10 rats/sex/dose for 14 weeks; diets prepared weekly; hematology, clinical chemistry and urinalysis at termination; no dose-related clinical signs or effect on eyes; **possible adverse effect indicated**; **hematopoietic changes & increased serum bilirubin at 200 and 800 ppm**; **fatty hepatocellular necrosis at 800 ppm**; NOEL = 50 ppm (4.2 - 4.4 mg/kg/day, hematopoietic changes); study not evaluated for acceptability because it does not conform to any SB-950 test type; study deficient in general areas (MTD not reached, no GLP) no worksheet. Davis, 01/15/88.

011 982454 "Oral Three-generation Study in Wistar Rats" (Janssen Pharmaceutical, no. 616, 11/18/75) Imazalil, R 23979, batch A 10/2, 99.5% from response of registrant; used as a wettable powder of 50%; fed in the diet to 20 females per group at 0, 5, 20 or 80 mg/100 gm food and stated to be approximately 0, 5, 20 and 80 mg/kg/day; on days 6-15 of pregnancy of the 1st and 2nd generations and from weaning to 3 months and from day 1 to 21 of pregnancy at which time they were sacrificed and the fetuses examined - radiographic exams of control and high dose fetuses; males not dosed; no adverse effect claimed, no effect on body weight of pregnant females in any generation, food consumption approximately the same in all groups and generations. The summary data, however, indicated the % of stillborn fetuses was increased several fold in the first and second generations but not in the third. No maternal toxicity is reported at this dose. Apparent systemic, maternal NOEL $\geq$ 80 mg/100 gm food, fetotoxic

NOEL = 20 mg/100 gm food. Unacceptable (protocol, no historical data for terata or reproductive parameters, radiologic exam). (Apostolou, 7/19/85, Gee, 11/86.)
EPA 1 liner: Minimum. Reproductive NOEL = 80 mg/kg/day (HDT), systemic NOEL = 80 mg/kg (HDT).

011 982456 "Oral Male and Female Fertility Study in Wistar Rats (Segment I)". (Janssen Pharmaceutical, No. 598, 3/3/77). Imazalil, R23979, lot C 03/1, approximately 99%, as 50% wettable powder, RE II.108; fed in the diet to 20/sex/group at 0, 5, 20 or 80 mg/kg beginning in males 60 days before mating and 14 days for females through gestation; apparent NOEL $\geq$ 80 mg/kg; unacceptable (protocol not acceptable for reproduction study, test article not characterized, no analysis of diet, doses not justified), no adverse effect reported. This contrasts with the other studies where some adverse effects on fetal/pup survival were reported. (Apostolou, 7/19/85, Gee, 11/86).
EPA 1-liner: Minimum. Fertility NOEL = 80 mg/kg/day.

058 074001, "Six-Month Oral Toxicity Study with Imazalil Base-R 23979 in Rats", (Civo Institutes TNO, Report No. V 83.186/220555, September 1983). ZR 23979, purity 98.1%; fed in the diet at nominal concentrations of 0, 25, 100, or 400 ppm to 10 Wistar SPF rats/sex/group for 6 months. Increased relative weights in some organs at 400 ppm, particularly kidney and liver in females and kidney in males. No histopathological effects noted at any dose for either sex in any organ or tissue. The nominal systemic NOEL = 100 ppm based on decreased weight gain in males at 400 ppm; nominal NOAEL $\geq$ 400 ppm (no biologically significant chronic or oncogenic findings); no individual data. No further data needed for this study at this time. Supplementary to # 047215. (Kishiyama, 9/26/89 and Gee, 11/1/89)

METABOLISM:

073 113033 "General metabolism of imazalil in the rat," (Mannens, G., Van Leemput, L. and Heykants, J., Janssen Research Foundation, 6/10/91; Report #: R 23979/FK1116). Imazalil technical (purity = 98.7%) and ^{14}C-imazalil (117.1 uCi/ml; purity = 99.9%) was administered to 4 groups of 6-8 Wistar rats. **Group A:** 5 rats/sex & reserve group I (1/sex) were injected in

tail vein with 1.25 mg ^{14}C-imazalil/kg (single dose). **Group B:** 5/sex were dosed by gastric intubation at 1.25 mg ^{14}C-imazalil/kg (single dose). **Group C:** 5/sex & reserve group K (3/sex) were dosed by gastric intubation at 1.25 mg imazalil/kg/day for 14 days. At 24 hours after the last unlabelled dose, 5/sex received a single oral dose of ^{14}C-imazalil at 1.25 mg/kg. **Group D:** 5/sex & reserve group L (1/sex) were dosed by gastric intubation at 20 mg ^{14}C-imazalil/kg (single dose). At 96 hours post-dosing (Groups B, C & D) were sacrificed for tissue collection. Group A & reserve rats (those not used) were sacrificed & disposed of. **RESULTS:** Distribution showed that after 96 h only 1% of ^{14}C-imazalil was recovered in tissues and carcass. There was a dose-response in tissue levels of compound but there was no accumulation after multiple dosing. There were no sex differences. Approximately 50% of tissue ^{14}C-imazalil was recovered in liver. 96 h after gavage, levels in liver were approximately 20 times higher and kidney, lung and adrenals 4-10 times higher than corresponding blood levels. All other tissues examined had concentrations of ^{14}C-imazalil $\leq$ that of blood, with none detected in brain. By all routes and methods of administration, the majority (approximately 90%) of radioactivity was excreted within 24 h (primarily in urine & slightly higher in females). Metabolism showed unchanged imazalil in all urine samples (measured by radio-HPLC) was below the level of detection. There were more than 25 metabolites of ^{14}C-imazalil in the 0-24 h pools. Few, if any differences in metabolite patterns between sexes or among the groups were detected. Methanolic extracts of radioactivity from feces were 62% in males & 70% in females (similar in all groups) & feces also showed 25 metabolites detected at 0-24 h. Unchanged imazalil excreted in urine was 0.07% of the dose. Imazalil was considered to have high bioavailability. This study is supplemental but not acceptable as a metabolism study (Clinical signs not described at the high dose; no justification in the report for 20 mg/kg as the high dose; no description of randomization procedure used in this study). M. Silva, 11/16/93.